

Mono-Methyl Histone H3 (Lys4) Antibody

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rev. 04/25/16

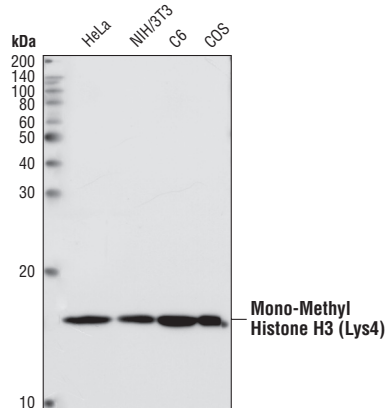
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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-IC Endogenous	H, M, R, Mk, (Z, X)	17 kDa	Rabbit**

Background: The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (1). Histone methylation is a major determinant for formation of active and inactive regions of the genome and is crucial for proper programming of the genome during development (2,3). Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4) (4). In contrast, a more diverse set of histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the *Drosophila* Su[var]3-9, Enhancer of zeste and Trithorax proteins. Lysine methylation occurs primarily on histones H3 (Lys4, 9, 27, 36, 79) and H4 (Lys20) and has been implicated in both transcriptional activation and silencing (4). Methylation of these lysine residues coordinates the recruitment of chromatin modifying enzymes containing methyl-lysine binding modules such as chromodomains (HP1, PRC1), PHD fingers (BPTF, ING2), tudor domains (53BP1) and WD-40 domains (WDR5) (5,6,7,8). The recent discovery of histone demethylases such as PADI4, LSD1, JMJD1, JMJD2 and JHDM1 has shown that methylation is a reversible epigenetic mark (9).

Specificity/Sensitivity: This antibody detects endogenous levels of histone H3 when mono-methylated on Lys 4. The antibody shows slight cross-reactivity with histone H3 when di-methylated on Lys4, but does not cross-react with non-methylated or tri-methylated Lys 4. In addition, the antibody does not cross-react with methylated histone H3 Lys9, Lys27, Lys36 or methylated histone H4 Lys20.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to the amino terminus of histone H3 in which lysine 4 is mono-methylated. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of lysates from HeLa, NIH/3T3, C6 and COS cells, using Mono-Methyl Histone H3 (Lys4) Antibody.

Background References:

- (1) Peterson, C.L. and Laniel, M.A. (2004) *Curr. Biol.* 14, R546–R551.
- (2) Kubicek, S. et al. (2006) *Ernst Schering Res. Found. Workshop*, 1–27.
- (3) Lin, W. and Dent, S.Y. (2006) *Curr. Opin. Genet. Dev.* 16, 137–142.
- (4) Lee, D.Y. et al. (2005) *Endocr. Rev.* 26, 147–170.
- (5) Daniel, J.A. et al. (2005) *Cell Cycle* 4, 919–926.
- (6) Shi, X. et al. (2006) *Nature*, Epub ahead of print.
- (7) Wysocka, J. et al. (2006) *Nature*, Epub ahead of print.
- (8) Wysocka, J. et al. (2005) *Cell* 121, 859–872.
- (9) Trojer, P. and Reinberg, D. (2006) *Cell* 125, 213–217.

Swiss-Prot Acc. # P68431

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:25
Immunofluorescence (IF-IC)	1:200

For application specific protocols please see the web page for this product at www.cellsignaling.com.

Please visit www.cellsignaling.com for a complete listing of recommended companion products.

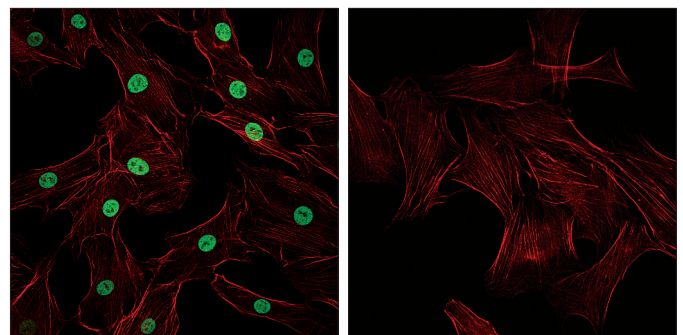
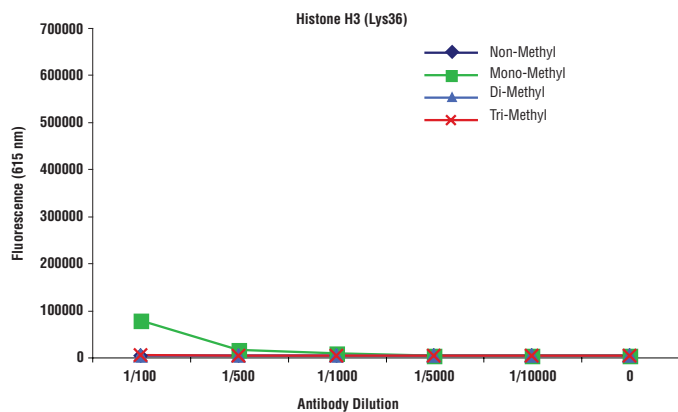
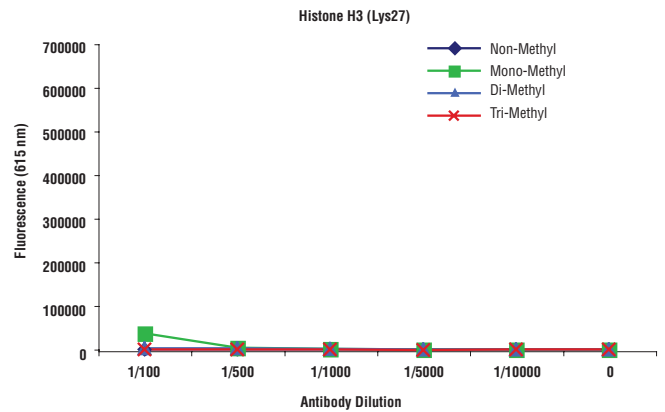
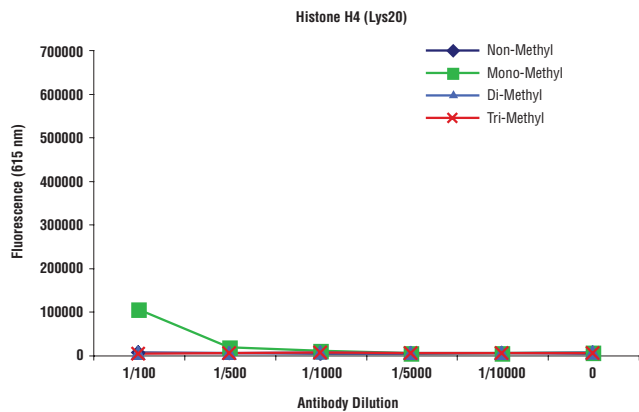
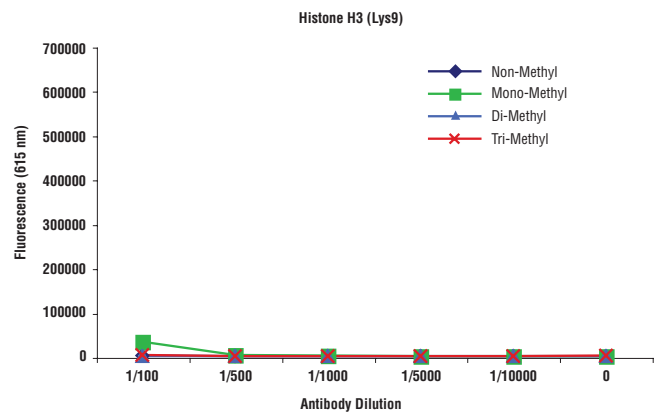
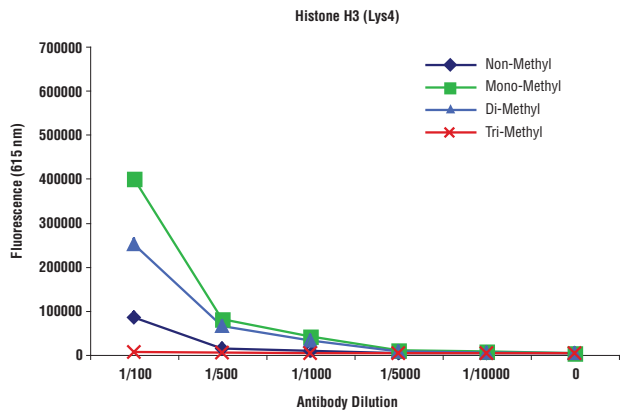
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.



Mono-Methyl Histone H3 (Lys4) Antibody specificity was determined by peptide ELISA. Each graph depicts a titration of this antibody and the corresponding reactivity toward the non-methyl, mono-methyl, di-methyl and tri-methyl states of the indicated histone H3 or H4 lysine residue.

Confocal immunofluorescent images of NIH/3T3 cells labeled with Mono-Methyl Histone H3 (Lys4) Antibody (green, left) compared to an isotype control (right). Actin filaments have been labeled with Alexa Fluor[®] 555 phalloidin (red).